THE UNIVERSITY OF ALBERTA

DETERMINATION OF THE RATE OF FORMATION OF THE NICKEL COMPLEX OF PHEOPHYTIN a

A THESIS

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BY

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ABSTRACT

The nickel complex of pheophytin may be a possible intermediate in the formation of petroleum porphyrins from chlorophyll. It is characterized in diethyl ether by a bright blue-green color with major absorption maxima at 410 and 635 mu in the visible range. The rate of formation of the nickel complex from nickel acetate and pheophytin was studied in methanol yielding the rate expression

$$\frac{dx}{dt} = k\ (\text{ Nickel acetate })^{0.7}\ (\text{ Pheophytin })^{0.5}$$

The reaction was investigated over the temperature range 74 to 115°C and the rate constant for this system, for time measured in seconds, is given by the equation

$$k = 3.9 \times 10^{9}\ e^{-23.0 \times 10^{3}/RT}$$

Extrapolation of the log k vs $\frac{1}{T}$ plot to sedimentary temperatures indicated that the formation of the nickel complex of pheophytin as an intermediate process in the formation of petroleum porphyrins from chlorophyll, is possible in the formation of crude oil provided that adequate supplies of nickel are available.

ACKNOWLEDGEMENTS

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INTRODUCTION

Very little is known of the mechanics of the formation of crude oil from its source materials to oil as it is found today. To investigate this mechanism a technique must be developed which will clearly define the conditions necessary for oil formation. This technique must involve a knowledge of both the original source materials of the oil and the chemical constituents of petroleum. It is generally accepted that there is a possibility of the occurrence in probable source materials of compounds which are almost identical with some of those found in crude oil. One specific example of such a relationship is the presence in crude oil of porphyrin molecules which are closely related to porphyrin-like chlorophyll in living plants. A study of the development of petroleum porphyrins from chlorophyll should provide a marker technique which would indicate the circumstances under which oil must be formed.

The American Society for Testing Materials defines crude petroleum as " a naturally occurring mixture consisting predominantly of hydrocarbons, and/or of sulphur, nitrogen and/or oxygen derivatives of hydrocarbons, which is removed from the earth in liquid state, or capable of being so

removed " (1).

The monumental work of Rossini and others (2) on Research Project 6, supported by the American Petroleum Institute, produced much information on the hydrocarbon components of one crude oil. In twenty-seven years of research 141 hydrocarbons have been quantitatively isolated from one of the Oklahoma crude oils. This group of hydrocarbons, representing about 44% of the oil, was made up largely of n-paraffins, alkyl cyclo-pentanes, alkyl cyclo-hexanes and alkyl benzenes. The conventional techniques of Rossini have been extended considerably. O'Neal and Wier (3) and Lumpkin and Johnson (4) using new mass spectrometric techniques identified hydrocarbons having molecular weights of 600 grams or higher. Kenny and Meinschein (5), using chromatography to obtain fractions of crude oils, substantiated to a considerable degree by mass spectrometry other evidence that the most numerous of the molecules making up crude oil are ring systems with many alkyl substituents.

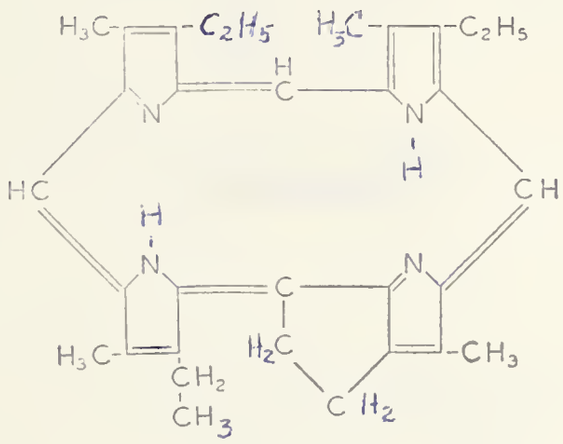
Crude oils, as outlined by the A. S. T. M., also contain a variety of non-hydrocarbon materials (6). Among these are oxygen, sulphur and nitrogen derivatives of hydrocarbons. The oxygen content is less than 2% by weight (7) with the oxygen accommodated predominantly in cyclo-pentyl acids and in lesser quantities in phenols, carboxylic acids and acid anhydrides. Of the sulphur derivatives of hydro-

carbons, compounds such as mercaptans, alkyl sulphides, cyclic sulphides, free sulphur and hydrogen sulphide are found. Generally speaking most crude oils contain less than 2% sulphur by weight. The nitrogen content of crude oils is usually less than 1% by weight. Bailey and co-workers (8) have isolated quinolines and pyridines. Non-basic compounds reported in crude oils indicate the presence of carbazoles, pyrroles and indoles (9). Porphyrins, which have a nitrogen content of about seven per cent, have been reported in crude oils as reviewed by Dunning (10).

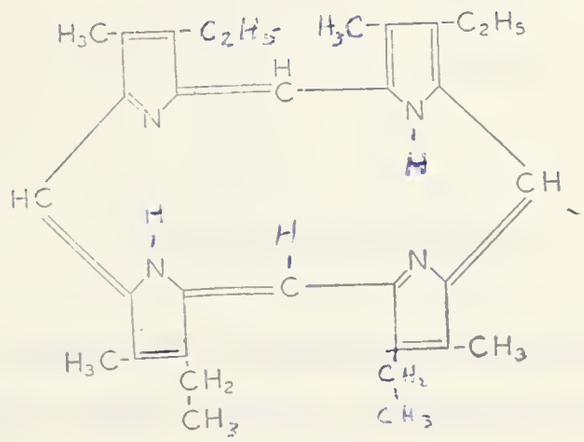
The ash content of crude oils varies depending on the character of the crude oil. It varies directly with the specific gravity of the oil. Several other properties of crude oils vary in a similar manner - vanadium, nickel, sulphur and asphaltene contents (11, 12). At least fifteen metallic constituents have been observed in crude oil of which the most abundant are vanadium and nickel.

It is generally accepted that porphyrins combine readily with metals and account for part of the total ash content of crude oils (10, 11, 12, 13, 14, 15, 16, 17, 18). Treibs (17) found evidence of complex salts of porphyrins in oil shales. Ether extracts gave spectra with absorption maxima in the visible region corresponding to copper, zinc and magnexium salts of porphyrins. Evidence was noted of other metal-porphyrin complexes in much

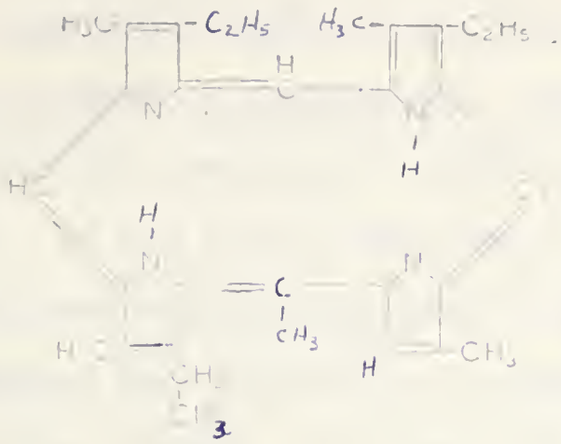
smaller quantities. Treibs (18) reported vanadium present in oil shales as vanadium complexes of deoxophyllerythroetioporphyrin, meso-etioporphrin, etioporphyrin and deoxophyllerythrin. It was suggested that the source of these porphyrins was chlorophyll from living plants.



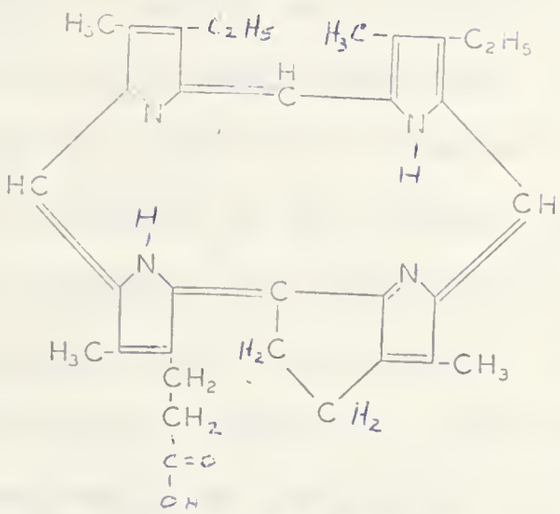
Deoxophyllerythroetioporphyrin



Meso-etioporphyrin



Etioporphyrin



Deoxophylloerythrin

These porphyrin complexes are decomposed only by the strongest acids. This is stated as the reason for the vanadium porphyrin complexes collecting and remaining in crude oil. Glebovskaya and Volk'enshtein (19) reported

iron, nickel and vanadium complexes of substituted hydrocarbons in a number of petroleums and bitumens. The spectra of these metal complexes indicated absorption spectra somewhat similar to that of chlorophyll. Sugihara and McGee (20) reported the presence of nickel porphyrin complexes in gilsonite. It was proved that the porphyrin involved was deoxophylloerythroetioporphyrin or an isomer. The authors suggested that gilsonite is derived largely, if not entirely, from plants. In view of the fact that petroleum porphyrins are believed to have developed from chlorophyll it appears reasonable that the conversion of chlorophyll to petroleum porphyrins could be used as a marker technique to define more clearly the development of crude oil from its source material. This could be done by establishing the conditions required to change from one compound to another in the overall chain of alterations in the marker compounds and subsequently arriving at the conditions necessary for oil formation. The present investigation deals with one reaction in that system of reactions - the metal complexing of pheophytin (one of the first degradation products of chlorophyll).

It is evident that crude oil is a complex mixture of hydrocarbons and oxygen, sulphur and nitrogen derivatives of hydrocarbons. Any realistic theory of source materials of petroleum and its subsequent alteration to petroleum must recognize this multitudinous number of

compounds with its accompanying suite of very special pigmented, metal-complexed compounds.

CHLOROPHYLL AND ITS DEGRADATION PRODUCTS

Fischer (21) and Steele (22) have written excellent reviews proving the structures of chlorophyll and some of its degradation products. Willstatter and Stoll (23) were among the first investigators to attempt a procedure of this nature.

Chlorophyll a and chlorophyll b are the two main green pigments in living plants. Chlorophyll a accounts for about eight percent of the leaf material and chlorophyll b about one-third that amount. More attention has been directed to the a series because of the abundance of chlorophyll a in plants.

Chlorophyll a is the magnesium complex of 1, 3, 5, 8 - tetramethyl -4-ethyl-2-vinyl-9-oxo-10-carbomethoxy-phorbin-7-propionic acid phytyl ester. It has a molecular weight of 893.5 grams. Its absorption maxima in ether are 660, 613, 577, 531, 498, 429 and 409 mu* (Figure 1). The ether solution is blue-green in color and has a red fluorescence.

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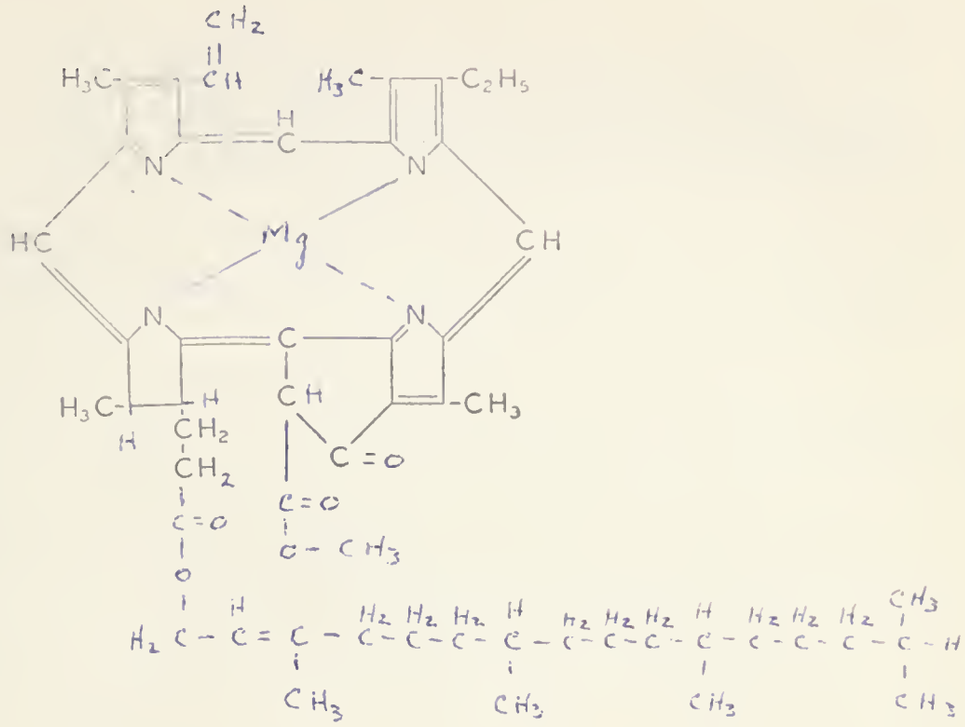
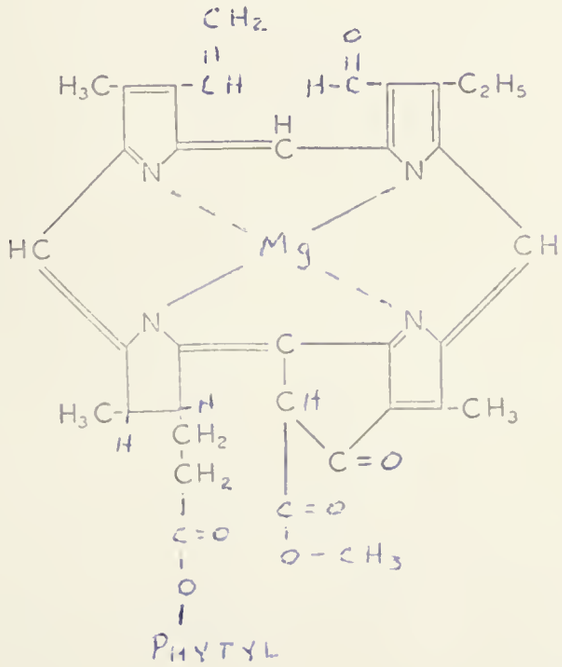
* The English letter u is used in place of the Greek letter μ for convenience to identify the wavelength.



Chlorophyll a



Chlorophyll b

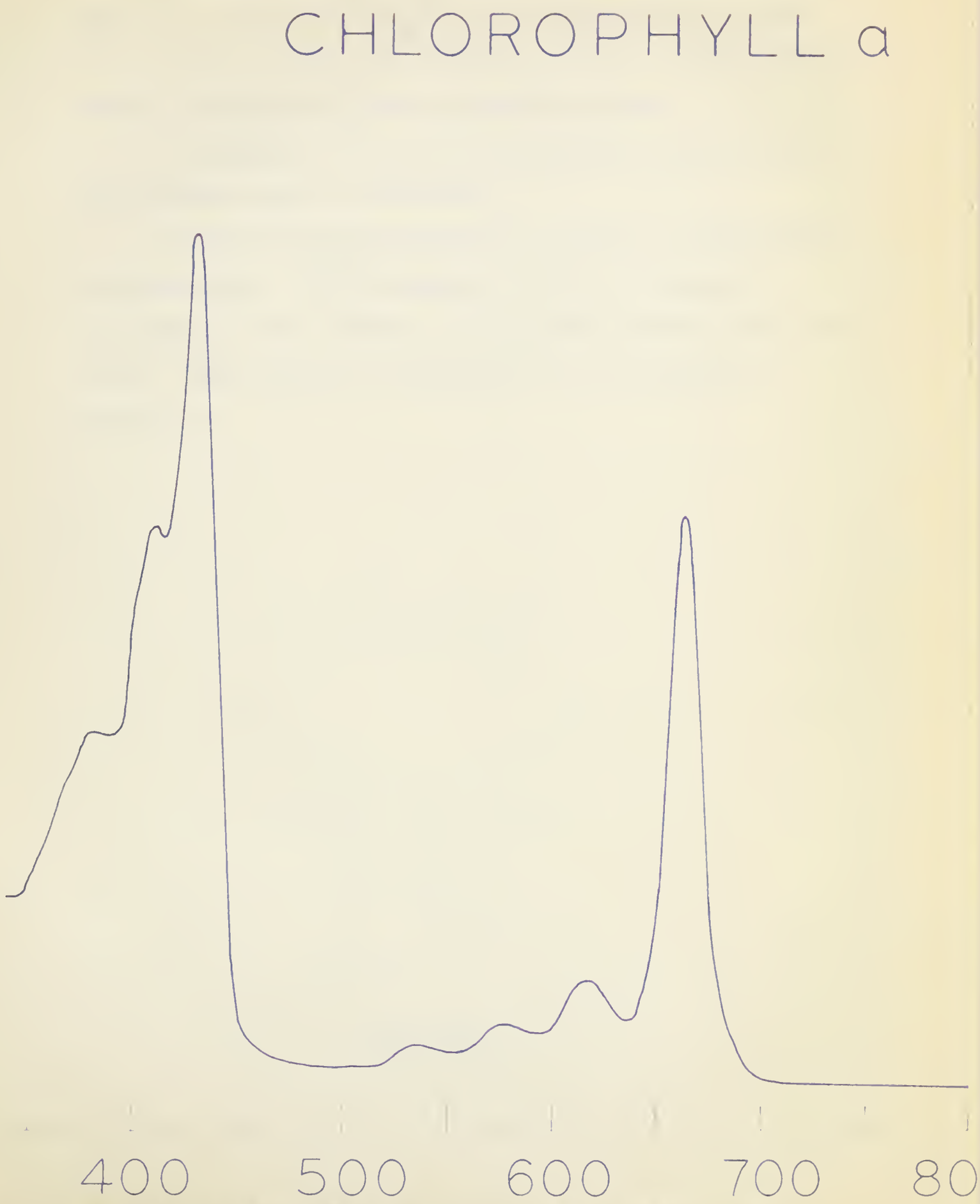
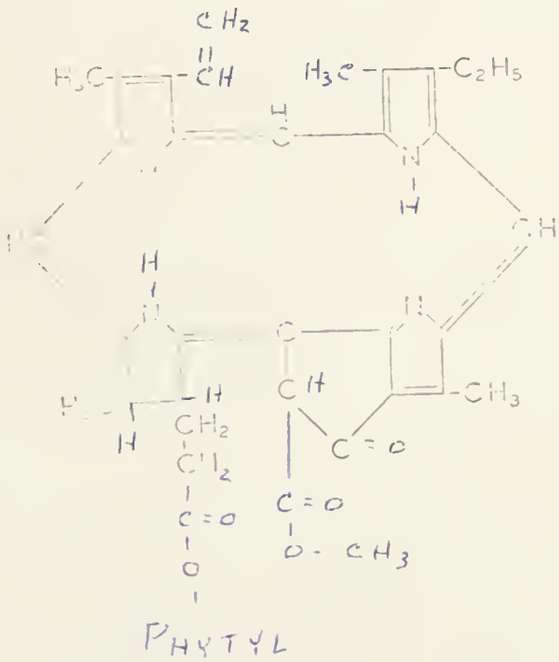


Fig. 1. Absorption spectrum of chlorophyll a in diethyl ether.

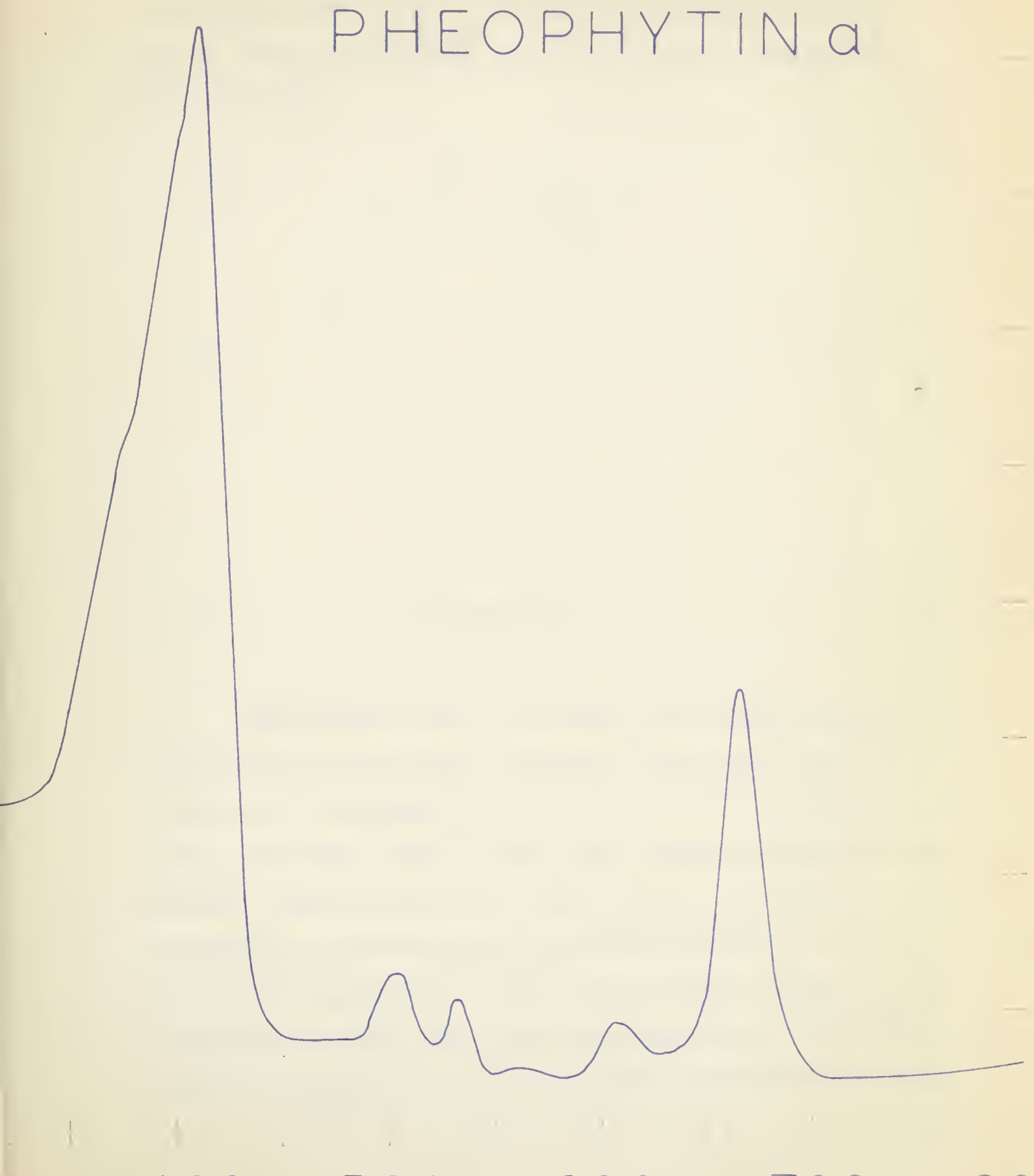
Chlorophyll b is virtually identical with chlorophyll a. The only difference is in the group on carbon three of the molecule, chlorophyll a having a methyl group whereas chlorophyll b has an aldehyde group.

Throughout the chain of degradation products only the a series will be discussed.

The usual first degradation product of chlorophyll is pheophytin. It is obtained by acid hydrolysis of chlorophyll which removes the magnesium ion from the molecule. The spectrum of pheophytin is illustrated in Figure 2.

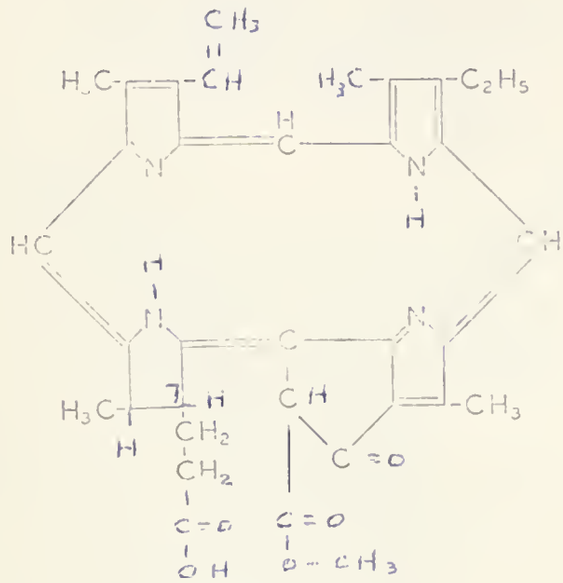


Pheophytin



g. 2. Absorption spectrum of pheophytin a in diethyl ether.

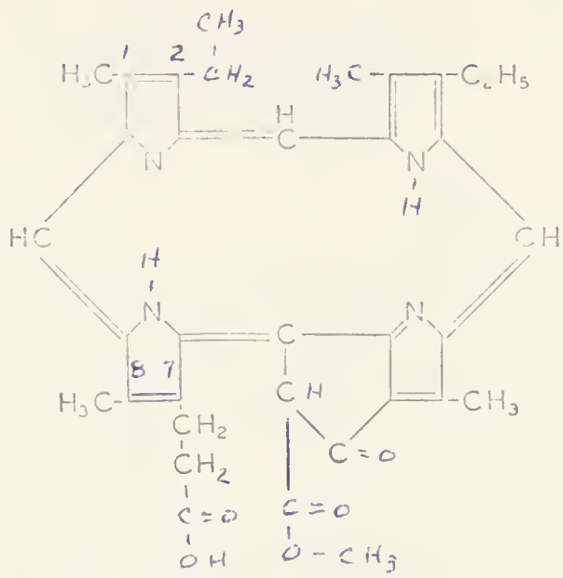
Pheophorbide is derived from pheophytin by further acid hydrolysis. This treatment hydrolyzes the phytyl group from the propionic acid side chain at carbon 7 of the molecule.



Pheophorbide

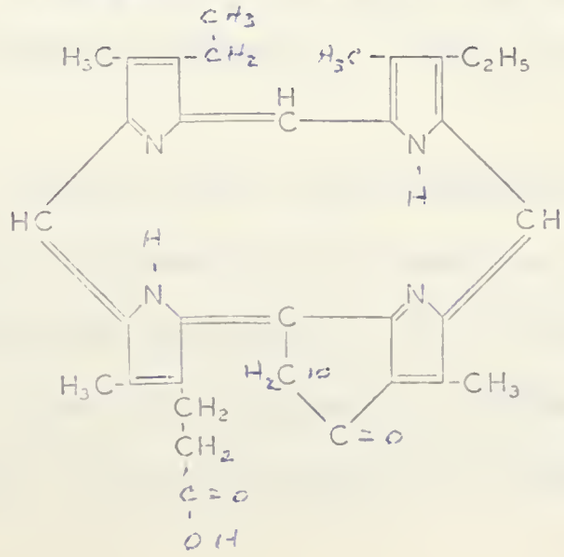
When pheophorbide is treated with hydriodic acid and acetic acid at 50°C reduction of the vinyl group at carbon two is accompanied by dehydrogenation at carbons seven and eight. This is the step in which porphyrins are formed. Chlorin pigments, which include chlorophyll, pheophytin and pheophorbide are also referred to by the term dihydroporphyrins. There is a distinct change in the absorption spectra here. Chlorin pigments are characterized by a maximum about 650 mu whereas the porphyrins have

maxima in the visible range at wavelengths shorter than 600 mu. The compound resulting from the reducing action of hydriodic acid and acetic acid on pheophorbide is pheoporphyrin.



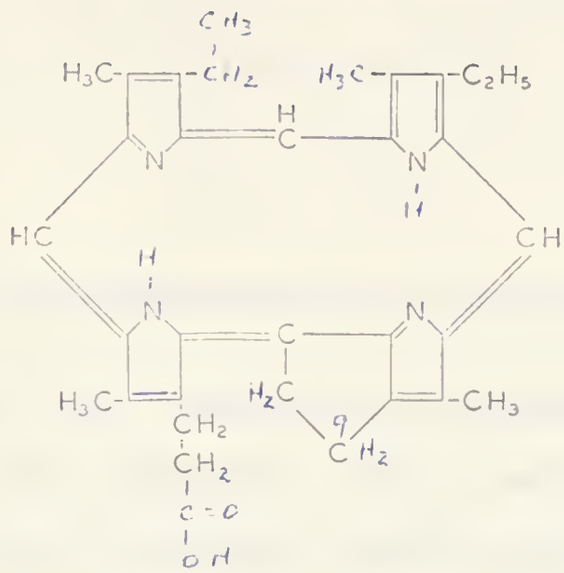
Pheoporphyrin

The conversion from pheoporphyrin to phylloerythrin is accomplished by the action of hydrobromic acid and acetic acid at 50 - 60°C on pheoporphyrin. This treatment results in de-esterification and decarboxylation at carbon 10 of the porphyrin molecule.



Phylloerythrin

When phylloerythrin is heated with hydrobromic acid and acetic acid at 180°C the oxo- group at carbon nine is reduced producing deoxophylloerythrin.

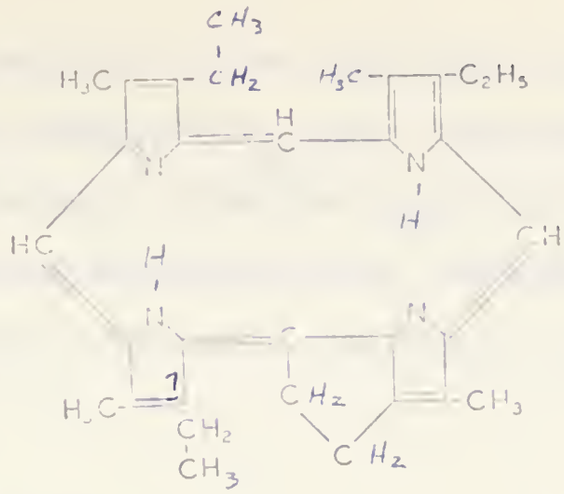


Deoxophylloerythrin

The final alteration in the chain of degradation products of chlorophyll is brought about by heating deoxophyllerythrin with soda lime at 300°C. Deoxo-phyllerythroetioporphyrin is the resulting product. The difference between these two compounds is in the side chain at carbon seven. The soda lime treatment brings about decarboxylation of the side chain at carbon seven.

This compound has been identified in petroleum most commonly complexed with nickel or vanadium.

Willstatter and Stoll (23) set up chemical procedures for obtaining degradation products of chlorophyll. The procedures outlined methods for preparation and



Deoxophylloerythroetioporphyrin

separation of these compounds and indicated the chemical structure of the compounds. Due to lack of modern spectrometer facilities the visible spectra were not reported precisely.

The following observations have also been reported regarding the conversion of chlorophyll to petroleum porphyrins:

1. Pheophytin a is converted to deoxophylloerythroetioporphyrin when it is heated for several hours in a petroleum medium at 300 - 360°C (24),

2. The nickel complex of pheophytin a is converted to a nickel-porphyrin complex when it is heated in 35% potassium hydroxide in ethylene glycol for twenty minutes at 200 - 250°C (25),

3. The metal ions in vanadium-porphyrins, nickel-porphyrins, and in the nickel complex of pheophytin have all been complexed under relatively mild conditions in the laboratory (18, 25, 26, 27, 28).

The chemistry for the conversion of chlorophyll to petroleum-type porphyrins is well established. Studies must be undertaken to determine the rates of reaction throughout the system of alterations and the rates of formation and stabilities of the metal complexes of each of the compounds.

ORIGIN OF CRUDE OIL

Two general mechanisms have been proposed for the origin of oil. One is a semi-classical approach proposing that oil was derived from many types of compounds such as fats, proteins and carbohydrates containing carbon and hydrogen derived from living organisms as summarized by Trask (29) about thirty years ago, and more recently by Stevens (6). The suggestion was that these compounds were very extensively altered in the formation of crude oil. The second mechanism has arisen recently as a proposal for a preferential preservation and mild conversion of hydrocarbon-like substances which occur in minute amounts in plants as outlined by Meinschein (30, 31) who reported the presence, in crude oils, of compounds only slightly different from trace hydrocarbons in living plants. Hunt (32) and Smith (33) supported a theory between the two extremes and suggested that the process of oil formation probably involves both a degradation of raw organic matter and a preferential preservation and concentration of certain

hydrocarbons from the remains of plants and animals. It is interesting to note that Knebel (34) calculated the total volume of organic matter trapped in sedimentary rocks at 300,000 cubic miles whereas the total world crude oil reserves are estimated at only 23 cubic miles indicating that petroleum makes up only a minor part of the total organic matter.

Two general conditions are generally accepted as necessary for crude oil formation - reducing conditions and low temperatures. Chlorins, dihydroporphyrins, which occur in recent sediments have not been observed in sedimentary rocks or petroleum. They are replaced in these older deposits by metallo-porphyrins (nickel and vanadium compounds predominantly) and to a lesser extent by free porphyrins. Treibs (24) reported that reduction processes must take place to alter chlorophyll pigments to porphyrin pigments. Teodorovich (39) noted that the most favorable conditions for oil formation were reducing conditions, varying from strongly reducing to weakly reducing systems. Metallo-porphyrins were first isolated from sedimentary rocks and petroleum by Treibs (17, 18, 24, 26). Nickel porphyrins have been reported in deposits as old as the Precambrian age (35).

A low temperature history for the formation of crude oil is generally accepted. Treibs (24) reported the

presence of porphyrins in petroleum which are destroyed at temperatures about 250°C. Bailey (8) found complex nitrogen compounds in a California crude oil which are unstable above 200°C. Hodgson and Baker (33) studying decomposition of petroleum metallo-porphyrins found that vanadium and nickel porphyrins would survive for more than 10^9 years if they were continuously preserved in a petroleum medium at a temperature of 100°C.

PIGMENTS IN RECENT SEDIMENTS

Since it is generally accepted that crude oil arises from organic matter of plant organisms, it is advisable to examine the circumstances under which the organic matter collects. The productivity of organic matter on the Earth is centred in shallow or near-shore seas and it is here that the bulk of the organic debris accumulates. It is here, also, that the bulk of sedimentation from erosion of land areas takes place. Therefore it is reasonable that the origin of crude oil is likely to be located in recent sediments. Examination of such recent sediments have shown the presence of considerable amounts of organic matter (25, 29, 30, 31, 33, 37, 38, 40, 41).

The chemical nature of organic matter in recent sediments has been a question of geochemical interest for a long time. A number of workers have approached the problem by analysis for particular components. This approach is

relatively new. It dates from approximately forty years ago when cellulose was clearly demonstrated to have been almost perfectly preserved in lignites known to be more than twenty million years old (42, 43, 44). It is now known that a variety of biological substances have been preserved in a relatively intact manner for periods of several hundred million years in extreme cases (10, 41, 45, 46, 47). Vallentyne (47) suggested that a solution to the problem must come from data on the molecular composition of recent and fossil organic matter.

Of particular interest in the present study is the occurrence of chlorophyll and its immediate degradation products - the chlorins. Corcoran (48) obtained evidence for the presence of pheophytins a and b and pheophorbides a and b in marine sediments. They were the dominant pigments in the sediments studied. Brown (Vallentyne, 47) isolated pheophytins a and b, chlorophyllides a and b, pheophorbides a and b, methyl pheophorbide a, chlorophyll b and bacteriochlorophyll in surface and near-surface sediments of Little Round Lake, Ontario. The existence of chlorophyllides a and b in the sediments of this lake show that at least some of the chlorophyll molecules lose the phytyl group before the magnesium ion (44, 47). This is an anomaly because the usual degradation of chlorophyll produces pheophytin by losing its magnesium ion. Brown noted a tendency for pheophytins to be replaced by pheo-

phorbides in the deeper sediments of a core taken from the same lake. This tendency was also observed by Hodgson et al (25) in a core taken from North Cooking Lake, Alberta. Vallentyne and co-workers (38, 50) and Orr and Grady (57) observed sedimentary chlorophyll degradation products in sediments of some shallow lakes in Connecticut. The above authors and Orr, Emery and Grady (37) found marked decreases in the amounts of degradation products with increasing depth of near-surface sediments.

A short summary of pigments in recent sediments may be made in the following manner:

1. Chlorophyll a, the most abundant green pigment in plants, is of extremely rare occurrence in sediments,
2. Chlorophyll b, which tends to lose its magnesium ion less readily than chlorophyll a, is found in at least some near-surface sediments,
3. With the exception of chlorophyll b and bacteriochlorophyll all the sedimentary green pigments appear to be transformation products of known chlorophylls,
4. Two initial degradation pathways appear common, the first more common than the second:

chlorophyll a $\longrightarrow$ pheophytin a $\longrightarrow$ pheophorbide a,

chlorophyll a $\longrightarrow$ chlorophyllide a $\longrightarrow$ pheophorbide a.

In general the chlorin pigments are predominant in recent sediments but there have been reports of the occurrence

of porphyrins which are very similar to, if not identical with, porphyrins in crude oil (25). Hodgson et al (25) found chlorin pigments in the uppermost layers of a core from North Cooking Lake, Alberta. In silts below an intervening blue clay layer a colored substance was observed which was apparently a single metal-porphyrin complex. This complex was identified as a vanadium complex. Similar occurrences have been reported by Fox et al (52, 53). The two porphyrins that are most abundant in older sediments (several million years old) are deoxophylloerythrin and its decarboxylation product deoxophylloerythroetioporphyrin (47). They occur most commonly in combination with either nickel or vanadium. The specific relation of these to chlorophyll a is evident from the presence of the five-member ring linking pyrrole group III with the γ- methene carbon.

A proposal has been made suggesting two possible mechanisms by which the pheophytin molecule is altered to the metal-porphyrin complexes found in crude oil (25, 51). First: pheophytin becomes complexed with the appropriate metal after which the metal complex of pheophytin is altered to form the metal-porphyrin complex. Second: pheophytin is altered to the free porphyrin which is subsequently complexed with the appropriate metal to a metal-porphyrin complex. Of the two proposed mechanisms for the

conversion of pheophytin to a metal-porphyrin complex, the present investigation deals with the first step of the first mentioned mechanism - the metal complexing of pheophytin.*

METAL COMPLEXES OF PHEOPHYTIN

Earlier investigators have found that metals will combine with pheophytin. Kuhn and Winterstein (54) were able to form the zinc complex of pheophytin by adding zinc dust and glacial acetic acid to a benzene solution of pheophytins a and b. Willstatter and Sjoberg (55) prepared the zinc complex of pheophytin a by the addition of an aqueous zinc acetate solution to a glacial acetic acid solution of pheophytin a. They also formed the same complex by heating a mixture of zinc acetate and pheophytin, both in pyridine, on a steam bath. The copper complex of pheophytin was prepared by Willstatter and Sjoberg (55) treating copper acetate and pheophytin in the same manner as that for the formation of the zinc complex. Rackow and Konig (56) attempted to form the metal complexes of pheophytin with magnesium and zinc. They mixed alcoholic solutions of pheophytin separately with magnesium chloride and zinc chloride salts. After fourteen days of standing

* In living plants chlorophyll a is the most abundant chlorin pigment exceeding its next most abundant pigment, chlorophyll b, by about three times. In this investigation attention was paid only to the a series. Further reference to either chlorophyll or pheophytin unless otherwise stated, should be interpreted as the a series compound.

the zinc complex of pheophytin had formed but no indication of a magnesium complex was observed. Addition of pyridine to the magnesium containing solution brought about complex formation in three weeks. The introduction of iron into the pheophytin molecule has been achieved by the addition of ferrous acetate in glacial acetic acid to a pyridine solution of pheophytin (57). Kukhtevich (58) made metal complexes of pheophytin using alcoholic or aqueous solutions of acetates, nitrates, chlorides and sulphates of bivalent copper, cobalt, zinc, cadmium and lead. Completion of the reaction was indicated by a green coloration. In general it was found that the metal-acetate salts possessed a much greater tendency to form complexes than the other anions. Lamort (59) was able to complex pheophytin with salts of gold, copper, zinc, iron, ammonia, sodium, potassium, beryllium, magnesium, calcium, aluminum, chromium, cobalt, platinum and palladium. No mention was made of the vanadium complex of pheophytin but Lamort (59) did make the observation that the nickel complex was not formed.

Metal complexes of chlorins are generally easy to differentiate from the non-metal-complexed chlorins by means of their visible spectra. Common to both types of compounds are two major maxima of absorption - one in the region about 400 mu and the other, of slightly less intensity, in

the region near 650 mu. The metal-free chlorins have three minor absorption peaks between the two major peaks whereas the metal-complexed chlorins have only one minor peak in this region. This difference has been attributed to an increase in the covalent character of the bonds between the pyrrole nitrogens and the central metal substituent (57).

A kinetic study of the rate of formation of the nickel complex of pheophytin was undertaken in the present investigation in an attempt to determine whether the reaction were geochemically feasible and to what extent one might expect to find the nickel complex in recent sediments. The results of a parallel investigation in this laboratory (25) demonstrated the likelihood of the reaction in recent sediments by reacting sediments with their indigenous pigments with a gross excess of nickel salt at elevated temperatures. The nickel complex of pheophytin has not been reported in undisturbed recent sediments but this may be due to its presence there in as yet undetectable quantities. The present investigation was undertaken in an attempt to study the reaction system more precisely. This was done by going to a homogeneous reaction system.

Other workers have been successful in forming metal complexes of pheophytin in a glacial acetic acid medium. The nickel complex of pheophytin had been prepared in this laboratory by the addition of nickel acetate to pheophytin

in a glacial acetic acid medium; however, to approach the true geochemical situation more closely it was necessary to study the reaction in a neutral medium. The pH of sediments is of course higher than that of a glacial acetic acid solution, the pH of sands and silts beneath a stagnant lake being in the range 7.4 to 8.6 (25). Other workers have reported similar values for other recent sediments (33, 41, 60). Methanol was chosen as the solvent due to the very low solubility of pheophytin in water.

In the determination of the rate of formation of the nickel complex of pheophytin, the nickel complex was first characterized and then the order of reaction with respect to each reactant was determined. In addition, the reaction rates were measured at several temperatures to determine the temperature dependence of the reaction.

EXPERIMENTAL

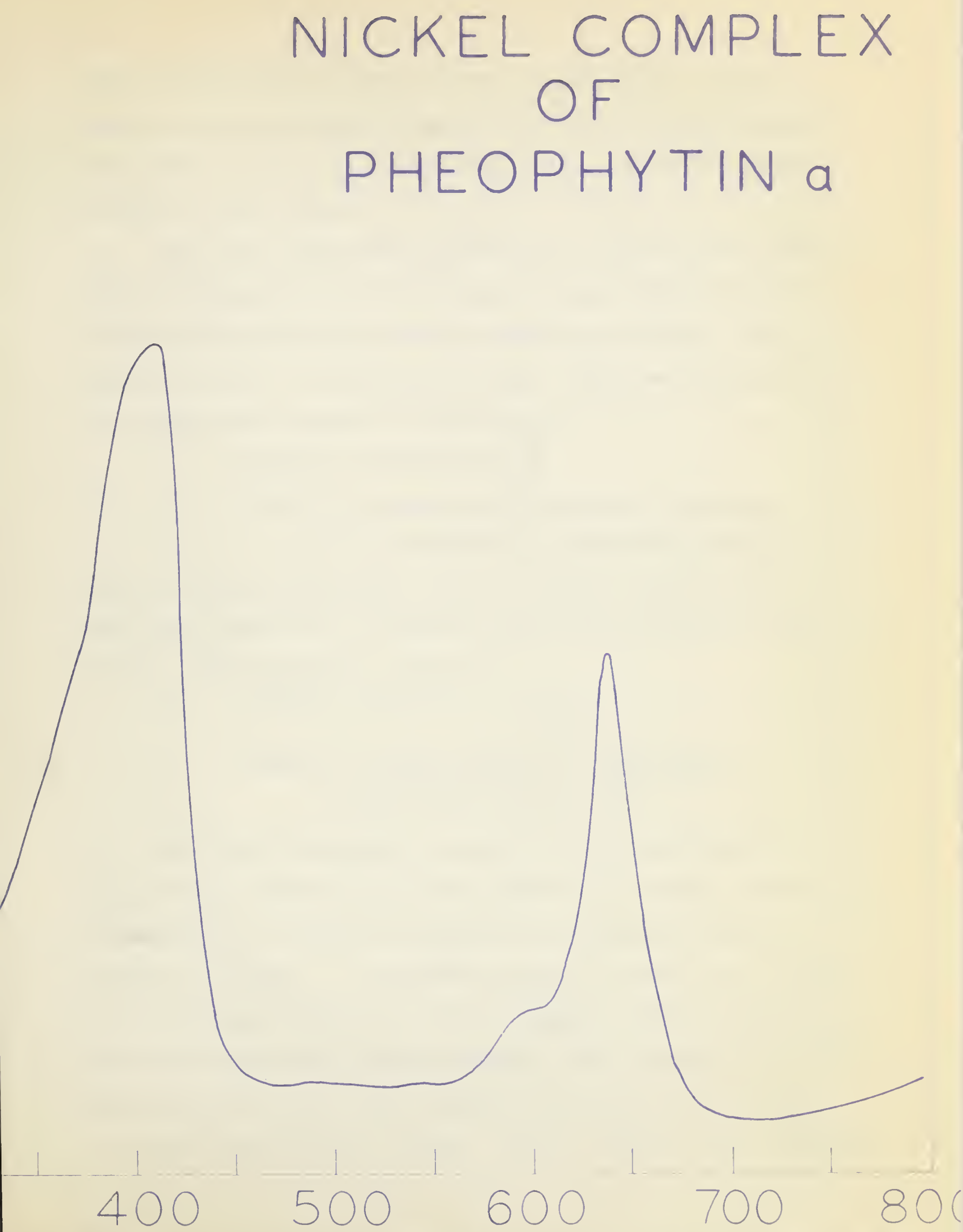
NICKEL COMPLEX OF PHEOPHYTIN

A stock solution of the nickel complex of pheophytin was prepared for characterization of the complex. The reactants used in the preparation were pheophytin and nickel acetate in methanol. Pheophytin was prepared from chlorophyll which had been obtained from spinach by acetone extraction and chromatographic separation as outlined by Zscheile and Comar (61). The chlorophyll was converted to pheophytin by the addition of methanolic-hydrochloric acid and further chromatographic separation as outlined by Livingstone et al (62). The resulting pheophytin was taken into solution with reagent grade methanol. Fisher certified reagent $Ni(C_2H_3O_2)_2 \cdot 4\ H_2O$ was dissolved in reagent grade methanol for use as a source of nickel ions in the reaction.

The nickel complex was prepared by heating pheophytin and nickel acetate in methanol at 115°C for 60 hours. Separation of the nickel complex from pheophytin and other remaining reactants and products was accomplished by solvent extraction and chromatographic separation. The reaction mixture was transferred to a separatory funnel containing twenty-five millilitres of distilled water and twenty-five millitres of reagent grade ether. The colored compounds passed into the ether layer. After washing with distilled water to remove water soluble materials the ether solution

was dried over anhydrous sodium sulphate to remove traces of water. To prepare the nickel complex of pheophytin and pheophytin for chromatographic separation the dried ether solution was evaporated to dryness on a steam bath under a stream of nitrogen. The residue was taken into solution in n-hexane and chromatographed on a sugar column which had been previously packed in n-hexane. The sugar used in the column was a commercially available powdered sugar, icing sugar, produced by the Canadian Sugar Factories Ltd., Raymond, Alberta. On elution with n-hexane the nickel complex moved down the column while the unreacted pheophytin remained at the top. In this manner a separation was effected to produce pure nickel complex or pheophytin. The chromatographic column was extruded and the nickel complex removed from the sugar with reagent grade diethyl ether. A spectrum over the range 325 to 800 mu was obtained of the ether solution with a Beckmann DK2 recording spectrophotometer.

The spectrum of nickel complex of pheophytin in diethyl ether in the visible region is characterized by two main absorption peaks, one at 410 mu and the other of slightly less intensity at 635 mu as shown in Figure 3. There are two other features of the spectrum which should be noted. There is a shoulder on the 410 mu peak at 395 mu and at 600 mu there is a minor peak of absorption. This spectrum is



. 3. Absorption of the nickel complex of pheophytin a in diethyl ether.

significantly different from that of pheophytin. In an ether solution pheophytin is identified by two major absorption peaks at 407 mu and 667 mu and minor absorption peaks at 505, 535 and 605 mu.

The molar extinction coefficient of the nickel complex of pheophytin was determined to relate optical density to the concentration of nickel complex in solution. The optical density is a measure of light radiation absorbed by the solution as defined by the relation

$$\text{Optical density} = \log \frac{I_o}{I}$$

where I_o = intensity of incident radiation
I = intensity of transmitted radiation

The Beer Lambert Law which relates optical density and concentration was used to calculate the molar extinction coefficient. The expression used was

$$\text{Optical density} = \frac{\epsilon}{c\ l}$$

where ϵ = molar extinction coefficient
c = concentration of solute, m/l
l = cell path length, cm

The molar extinction coefficient was determined by evaporating a quantity of stock solution of nickel complex of pheophytin in ether to dryness on a steam bath under a stream of nitrogen. A constant weight of residue was attained by continued heating of the residue for fifteen minutes after the sample had reached dryness. The resultant residue was weighed and taken into solution in increasing dilutions in reagent grade diethyl ether. Visible spectra were obtained

for each solution. Molar extinction coefficients for the complex in ether were calculated from the optical densities at 635 mu and concentrations for each dilution using the above equation. The coefficient was found to be constant over the range of concentrations at 7.0×10^4 litre $mole^{-1}cm^{-1}$ as reported in Table I. For comparison purposes molar extinction coefficients of some other chlorin and porphyrin pigments are reported in Table II.

The values of extinction coefficients for chlorophyll a, the nickel complex of pheophytin a and pheophytin a in ether, and deoxophylloerythroetioporphyrin in chloroform are 9.1×10^4, 7.0×10^4, 5.5×10^4 and 1.6×10^4 litre $mole^{-1}cm^{-1}$ respectively. Chlorophyll a is a very unstable compound and the porphyrin is a very stable compound. The molar extinction coefficients reported show chlorophyll with the largest value and the porphyrin with the smallest value. This may indicate a relationship between stability and the molar extinction coefficient. If so, the stability of the nickel complex of pheophytin would lie between that of chlorophyll and pheophytin. The nickel complex of pheophytin has not been reported in sediments but both pheophytin and deoxophylloerythroetioporphyrin have.

The nickel content of the nickel complex was determined spectrophotometrically according to the method used by Hodgson (11) adapted from that of Wrightson (63). In

TABLE I

Molar extinction coefficient

of the nickel complex of pheophytin

in

diethyl ether

Optical density, 410 mu	635 mu	Concentration, moles/litre	Molar extinction coefficients, 410 mu	635 mu
2.91	2.44	3.50×10^{-5}	8.31×10^{4}	6.98×10^{4}
1.47	1.23	1.75×10^{-5}	8.40×10^{4}	7.01×10^{4}
0.977	0.818	1.17×10^{-5}	8.35×10^{4}	6.98×10^{4}
0.736	0.616	0.89×10^{-5}	8.27×10^{4}	6.92×10^{4}
0.588	0.491	0.70×10^{-5}	8.41×10^{4}	7.02×10^{4}
		Average	8.4×10^{4}	7.0×10^{4}

TABLE II

Molar extinction coefficients of some chlorin and porphyrin compounds

Compound	Solvent	Absorption maximum, mu	Molar Extinction Coefficient, litre $mole^{-1}cm^{-1}$	Reference
Chlorophyll a	Diethyl ether	660	9.1×10^4	Zscheile and Comar (61)
Chlorophyll b	Diethyl ether	643	5.1×10^4	Zscheile and Comar (61)
Pheophytin a	Diethyl ether	667	5.5×10^4	Orr and Grady (51)
Pheophytin a	Chloroform	668	4.7×10^4	Orr and Grady (51)
Pheophytin a	Methanol	667	3.0×10^4	Livingstone et al (62)
Nickel complex of pheophytin a	Diethyl ether	635	7.0×10^4	This work
Vanadium complex of deoxophyllo-erythroetiopor-phyrin	Chloroform	575	1.6×10^4	Treibs (18)
Deoxophylloerythro-etioporphyrin	2N Hydro-chloric acid	552	1.6×10^4	Treibs (18)

this method the nickel was complexed with dimethylglyoxime and the intensity of the resulting colored solution was measured at 406 mu using a Hilger Uvispec spectrophotometer. The optical density of the solution was used to calculate the nickel concentration by a predetermined conversion factor. The theoretical nickel concentration of the nickel complex of pheophytin is 6.23%, the nickel concentration determined by the above method was 6.25%.

The characteristics of the nickel complex of pheophytin in reagent diethyl ether as determined in this investigation are:

1. The molar extinction coefficient is 7.0×10^4 litre $mole^{-1}cm^{-1}$,
2. The nickel content is 6.25%,
3. The solution is bright blue-green in color with a spectrum characterized in the visible range by two main absorption peaks at 410 and 635 mu, a minor absorption peak at 600 mu and a shoulder on the 410 mu peak at 395 mu. The absorption at 410 mu is slightly greater than that at 635 mu.

ANALYTICAL METHODS

The obvious method of measuring the concentration of the colored nickel complex, in studying the rate of formation of the nickel complex of pheophytin from nickel acetate

and pheophytin, was by light absorption methods. It was necessary, however, to separate the nickel complex from the unreacted pheophytin because of the close similarity of the spectra of the two compounds. Chromatographic methods were studied extensively. Some of the adsorbent systems investigated were powdered sugar, alumina, talc, magnesium oxide, calcium carbonate and magnesium carbonate. The solvents used in conjunction with adsorbents were n-hexane, n-heptane, petroleum ether, diethyl ether, carbon tetrachloride, chloroform, benzene, methanol, pyridine, iso-propanol and combinations of these solvents. Two separate systems gave indications of good separations and were investigated more closely. They were powdered sugar packed in n-hexane and calcium carbonate packed in benzene. The powdered sugar was commercially available icing sugar and the calcium carbonate was Fisher certified reagent calcium carbonate.

The powdered sugar in n-hexane system indicated a partial separation with the nickel complex moving down the column on elution with n-hexane. The pheophytin remained at the top. Investigation of the pheophytin layer indicated the presence of nickel complex resulting from an incomplete separation. Continued elution did not improve the separation and the use of stronger eluents moved the pheophytin down the column with the nickel complex.

The calcium carbonate-benzene system also indicated a separation when a benzene solution containing both pheophytin and the nickel complex were put on the column. On elution with benzene the nickel complex moved down the column very readily with the pheophytin remaining near the top of the column. When elution was continued both materials were eluted off the column at nearly equal rates.

Of the two systems which indicated possible separations the powdered sugar-n-hexane system was probably better than the calcium carbonate-benzene system. It is a slower moving system which results in a better separation. Application of a vacuum was necessary to move the eluent through the column.

It was observed that the nearly colorless eluate preceding the nickel complex of pheophytin off the column contained appreciable quantities of nickel apparently complexed with degradation products of pheophytin.

Unfortunately not all the nickel complex is separated from the pheophytin in this system and as a result, this system could not be applied to rate of formation study separations which required quantitative separations. The calcium carbonate-benzene system was reliable for separations only when small quantities of pheophytin relative to the nickel complex were present.

The two chromatographic systems which indicated

separations of the nickel complex of pheophytin from pheophytin appeared to be unsuitable for a study of the rate of formation of the nickel complex of pheophytin. Recourse was made to a separation method based on the acid solubility properties of the two compounds (23). Investigations were carried out to determine the concentration of hydrochloric acid necessary to remove either or both of the compounds from an ether solution. Results indicated that a concentration of 30% hydrochloric acid was necessary to remove pheophytin quantitatively from an ether solution as reported in Table III. A parallel study of the effect of hydrochloric acid on the nickel complex in an ether solution indicated a small transfer of the complex to the hydrochloric acid at an acid concentration level of 30%. Further investigation of this transfer of complexed material indicated a recovery of the complex by successive ether washes of the acid solution. All the nickel complex which had been removed by the acid was recovered in this manner. The acid separation and subsequent ether washing of the acid layer for recovery of the nickel complex are reported in Table IV.

The analytical method so developed involves the following steps for separation of the nickel complex from pheophytin:

1. A transfer of the pigments of the reaction mixture from methanol to ether.

TABLE III

Extent of removal

of pheophytin from ether solution

by means of hydrochloric acid

Concentration of acid, %	Extent of removal
10	None
20	None
25	None
28	Partial removal
30	Nearly complete
32	Complete removal

TABLE IV

Recovery of nickel complex from

a mixture of

pheophytin and nickel complex in ether

	Optical Density* at 635 mu	Recovery, %
Initial nickel complex in ether	0.460	100.0
Ether solution of mixture after pheophytin removal using three conc. hydrochloric acid washes	0.375	81.6
Final recovery after three ether washes of acid layer	0.456**	99.2

* All optical densities were adjusted to a standard volume.

** Ether washes of the acid layer were added to the solution containing the bulk of the nickel complex before obtaining the optical density.

2. A 30% hydrochloric acid extraction of the ether layer to remove the unreacted pheophytin from the nickel complex product.

3. An ether wash of the acid layer to recover nickel complex which passed into the acid layer.

APPARENT DEGREE OF DISSOCIATION OF NICKEL ACETATE IN METHANOL

A study was undertaken to determine the extent of dissociation of the nickel acetate in methanol. There are several methods which can be followed to determine the apparent degree of dissociation of a solute in a solvent. The method studied in this investigation was a conductometric method in which the electrical conductivity of the solute ions is measured. In this phase conductivity measurements were made on three concentrations of nickel acetate in methanol covering the same range as those to be studied in the rate of formation of the nickel complex of pheophytin, i. e. 5×10^{-5} to 5×10^{-4} moles per litre. All measurements were carried out using an Industrial Instruments Inc. Model RC16B1 Conductivity Bridge. With this instrument the resistance of the solution is measured between two plates of a cell with standard area and separation distance. The cell constant of the cell used was 0.1 centimeter - determined by dividing the area of the plates by the separation distance between the plates. The resistance of the solution depends on the

dimensions of the conductor and is related to them by the expression

$$R = \rho \frac{l}{A}$$

where R = resistance in ohms
ρ = specific resistance in ohm cms.
l = length of conductor in cms.
A = area of conductor in cm^2.

The reciprocal of the resistance is the conductance and the reciprocal of the specific resistance is the specific conductance or conductivity k. The units of conductance are reciprocal ohms or mhos and the units of the conductivity are reciprocal ohm cm.

The equivalent conductivities of the three concentrations of nickel acetate in methanol were determined at 25°C and are reported in Table V. Apparent degrees of dissociation were calculated from the equivalent conductivities at each concentration of solute and the equivalent conductivity at infinite dilution as reported by Thompson (64). These values are also reported in Table V and it was apparent that nickel acetate is not completely ionized in methanol with an apparent dissociation of about 25%.

RATE MEASUREMENTS

Rate measurements for the formation of the nickel complex of pheophytin from pheophytin and nickel acetate in methanol were carried out at four temperatures - 74°, 90°,

TABLE V

Apparent degrees of dissociation
of
nickel acetate in methanol at 25°C

Nickel acetate concentration, moles/litre	Measured resistance, ohms	Equivalent conductivity, mhos	Apparent * degrees of dissociation
0	27500	-	-
5×10^{-5}	14500	32.61	0.35
1×10^{-4}	10900	27.69	0.29
5×10^{-4}	3650	23.76	0.24
0	27500	-	-
5×10^{-5}	14550	32.35	0.34
1×10^{-4}	11150	26.67	0.28
5×10^{-4}	3850	22.33	0.24
0	27000	-	-
5×10^{-5}	14650	31.22	0.33
1×10^{-4}	11150	26.34	0.28
5×10^{-4}	3850	22.27	0.24

* Equivalent conductivity of nickel acetate at infinite dilution is assumed to be 94.4 mhos as for an aqueous solution (64)

103°C and 115°C. Reaction vessels were made of heavy wall glass tubing 13 mm outside diameter and 8 mm inside diameter. Each tube was cut to 30 cm in length and sealed at one end. A constriction was made in each tube about 5 cm from the open end to facilitate sealing of the tube.

All reactions were carried out at four temperatures in one of two types of heating vessels. Reactions at 115°C were performed in an insulated aluminum block furnace. Heating was provided by four 450-watt electric heaters controlled by a Minneapolis Honeywell Brown Pyrovane temperature controller to ± 0.3°C. The holes for the reaction tubes in the aluminum block were filled with nujol to give better heat transfer to the reaction tubes.

Reactions at 74°, 90° and 103°C were carried out in a commercially available pump-circulating constant temperature bath. The temperature was controlled with a Colora ultra thermostat which controlled the temperature to ± 0.01°C. The fluid used in the bath was a commercially available light mineral oil Wyrol J.

To enable determination of the order of reaction with respect to each reactant, three sets of experiments were carried out:

1. Nickel acetate 1×10^{-4} moles per litre and pheophytin 4×10^{-4} moles per litre,
2. Nickel acetate 1×10^{-4} moles per litre and pheophytin

1×10^{-4} moles per litre,

3. Nickel acetate 4×10^{-4} moles per litre and pheophytin 1×10^{-4} moles per litre.

This range of concentrations was studied because of the relatively low solubility of reactants in methanol and because the amounts of pheophytin available from the chlorophyll synthesis method was small. Each batch of pheophytin synthesized as previously described yielded about one hundred millilitres of 1×10^{-3} molar solution. The quantities used however were adequate for convenient spectrophotometric determination of the product.

For each ratio of nickel to pheophytin reactions were studied for three periods of time at each temperature. Reaction times were chosen to give low conversions to avoid side effects. For each set of runs a reaction mixture was prepared and five-millilitre aliquots were placed in each reaction tube.

Before sealing the tubes, they were attached to a vacuum system and the reaction mixture was frozen in liquid nitrogen. The vessel was evacuated and the mixture was melted. The reaction was frozen once more, re-evacuated to remove dissolved oxygen and carbon dioxide which may interfere in the reaction. After evacuating, the tubes were sealed off at the constriction, placed in the appropriate heating vessel and left there for the desired lengths of

time. At the completion of the reaction period the tubes were removed from the heating vessel and the reaction quenched by placing the reaction tubes in cold water.

Studies were made regarding the rates of heating of the reaction mixture to reaction temperature and the rates of quenching in both air and cold water. The results of these studies indicated that a period of about four minutes was required to raise the temperature of the reaction mixture from room temperature to reaction temperature, as shown in Figure 4. Quenching of the reaction by lowering the temperature to near room temperature required one minute if quenched in cold water but required a considerably longer period of time if quenched in air. Quenching was done in cold water to eliminate errors due to further reaction during the time necessary for air quenching.

After quenching, the tubes were opened and the reaction mixture transferred to a separatory funnel containing twenty five millilitres of distilled water and twenty-five millilitres of reagent grade diethyl ether. The mixture was shaken and the layers allowed to separate. Pheophytin and the nickel complex of pheophytin passed into the ether layer; methanol, any acetic acid formed and unreacted nickel acetate tended to pass into the water layer. The water layer was drained off. The ether layer was washed twice with distilled water to remove the water soluble materials. Twenty milli-

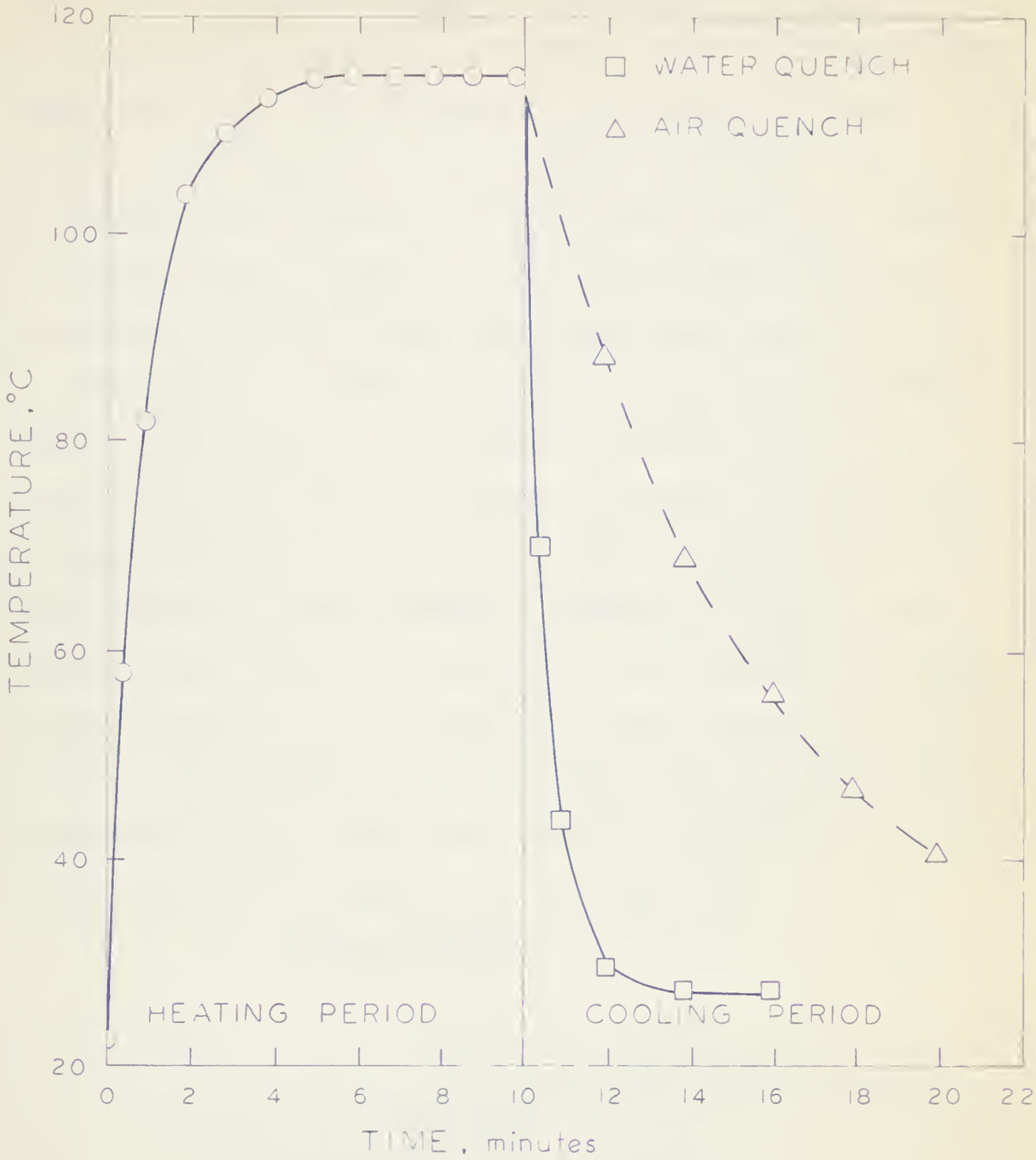


Fig. 4. Measurement of heating and cooling periods of reaction tubes to establish reaction times in rate of formation studies.

litres of concentrated hydrochloric acid were added to the ether layer to remove the unreacted pheophytin. The solution was shaken, the layers allowed to separate and the acid layer drawn off. The hydrochloric acid separation was repeated until the acid layer remained colorless. The acid washes were combined and washed with successive washes of diethyl ether to recover complexed material which had passed into the acid layer. The ether washes were added to the original ether layer containing the bulk of the nickel complex of pheophytin. The combined ether solution was washed twice with distilled water to remove any traces of hydrochloric acid. The acid free ether solution was dried over anhydrous sodium sulphate to remove the water. The volume of the solution was measured and a spectrum obtained of the solution. Optical densities were recorded for each sample. The concentration of nickel complex of pheophytin was calculated from the molar extinction coefficient and the optical density as expressed in the equation

$$c = \frac{\text{optical density}}{\epsilon \times l}$$

where c = concentration of complex in moles/litre
ϵ = molar extinction coefficient in litres/mole/cm
l = length of cell path in cm

In all cases the length of the cell path was 1.00 cm. Since ϵ is 7.0×10^4 for the nickel complex of pheophytin, the concentration is given by

$$c = \frac{\text{optical density}}{7 \times 10^4}, \text{ moles/litre}$$

THERMAL STABILITY OF THE NICKEL COMPLEX OF PHEOPHYTIN

Thermal stability of the nickel complex of pheophytin was investigated to determine whether any degradation took place during the course of the reaction. Aliquots of a stock solution of nickel complex of pheophytin in methanol were placed in reaction tubes and evacuated in the same manner as that for the rate of formation studies. The tubes were sealed, placed in the appropriate heating vessel and heated for periods of time greater than those used in the rate of formation studies. Degradation investigations were carried out at each of the four temperatures 74°, 90°, 103° and 115°C. No appreciable degradation of the nickel complex took place in these greater lengths of time as shown by the results in Table VI.

TABLE VI

Thermal stability of

the nickel complex in methanol

74°C		90°C		103°C		115°C	
Time, sec	Optical density*	Time, sec	Optical density*	Time, sec	Optical density*	Time, sec	Optical density*
0	0.341	0	0.358	0	0.358	0	0.341
2.394×10^6	0.316	2.88×10^4	0.357	2.16×10^4	0.357	1.44×10^4	0.338
2.700×10^6	0.298	5.76×10^4	0.343	4.32×10^4	0.349	2.88×10^4	0.326

* Optical density measurements were made at 635 mu and adjusted to a standard volume of solution for comparison purposes.

Maximum reaction times used in rate of formation studies at each temperature were: 74°C - 1.15×10^5 sec., 90°C - 2.88×10^4 sec., 103°C - 1.08×10^4 sec. and 115°C - 7.20×10^3 sec.

RESULTS

The rate of formation of the nickel complex of pheophytin from pheophytin and nickel acetate in methanol was studied at four temperatures - 74°, 90°, 103°, and 115°C and for various lengths of time ranging from 0.5 to 32 hours. Three ratios of nickel to pheophytin were used in the study to make possible a determination of the order of reaction:

1. 1×10^{-4} moles/litre nickel acetate and 4×10^{-4} moles/litre pheophytin.
2. 1×10^{-4} moles/litre nickel acetate and 1×10^{-4} moles/litre pheophytin, and
3. 4×10^{-4} moles/litre nickel acetate and 1×10^{-4} moles/litre pheophytin.

The extent of formation of the nickel complex corresponded to a conversion of total reactants to nickel complex in the range 4 to 26%. The detailed results are reported in Table VII.

The general form of the reaction would indicate a second order rate expression to fit the data, but a calculation of second order rate constants did not confirm this as shown in Table VIII.

The differential method was then used to determine the order of reaction with respect to each reactant. To determine the order of reaction with respect to nickel (α)

TABLE VII

Experimental data for the formation of the nickel complex of pheophytin

Concentration of reactants, moles/litre		74°C			90°C		
Nickel Acetate	Pheophytin	Time, sec	Optical density	Conc., moles/litre	Time, sec	Optical density	Conc., moles/litre
1×10^{-4}	4×10^{-4}	57600	0.372	0.0531×10^{-4}	10800	0.416	0.0594×10^{-4}
1×10^{-4}	4×10^{-4}	57600	0.390	0.0557	10800	0.479	0.0684
1×10^{-4}	4×10^{-4}	57600	0.343	0.0490	21600	0.752	0.1074
1×10^{-4}	4×10^{-4}	86400	0.664	0.0949	21600	0.900	0.1286
1×10^{-4}	4×10^{-4}	86400	0.546	0.0780	21600	0.752	0.1074
1×10^{-4}	4×10^{-4}	115200	0.848	0.1211	28800	1.104	0.1577
1×10^{-4}	4×10^{-4}	115200	0.738	0.1054	28800	1.131	0.1615
1×10^{-4}	1×10^{-4}	57600	0.181	0.0259×10^{-4}	10800	0.216	0.03085×10^{-4}
1×10^{-4}	1×10^{-4}	57600	0.193	0.0276	10800	0.220	0.03142
1×10^{-4}	1×10^{-4}	86400	0.290	0.0414	10800	0.218	0.03114
1×10^{-4}	1×10^{-4}	86400	0.292	0.0417	21600	0.403	0.0576
1×10^{-4}	1×10^{-4}	115200	0.428	0.0611	21600	0.415	0.0593
1×10^{-4}	1×10^{-4}	115200	0.414	0.0591	21600	0.365	0.0521
1×10^{-4}	1×10^{-4}				28800	0.496	0.0709
1×10^{-4}	1×10^{-4}				28800	0.495	0.0707
1×10^{-4}	1×10^{-4}				28800	0.483	0.0690
4×10^{-4}	1×10^{-4}	57600	0.523	0.0747×10^{-4}	10800	0.558	0.0797×10^{-4}
4×10^{-4}	1×10^{-4}	57600	0.636	0.0909	10800	0.544	0.0777
4×10^{-4}	1×10^{-4}	57600	0.600	0.0857	10800	0.544	0.0777
4×10^{-4}	1×10^{-4}	86400	0.794	0.1134	21600	1.000	0.1428
4×10^{-4}	1×10^{-4}	115200	1.850	0.2642	28800	1.274	0.1820
4×10^{-4}	1×10^{-4}	115200	1.215	0.1735			
4×10^{-4}	1×10^{-4}	115200	1.575	0.2250			

TABLE VII (Cont'd.)

Experimental data for the formation of the nickel complex of pheophytin

Concentration of reactants, moles/litre		103°C			115°C		
Nickel acetate	Pheophytin	Time, sec	Optical density	Conc., moles/litre	Time, sec	Optical density	Conc., moles/litre
$1x10^{-4}$	4×10^{-4}	3600	0.448	$0.0640x10^{-4}$	1800	0.636	$0.0909x10^{-4}$
$1x10^{-4}$	4×10^{-4}	3600	0.458	0.0654	1800	0.549	0.0784
$1x10^{-4}$	4×10^{-4}	3600	0.467	0.0667	1800	0.606	0.0866
$1x10^{-4}$	4×10^{-4}	7200	0.826	0.1180	3600	1.330	0.1900
$1x10^{-4}$	4×10^{-4}	7200	0.818	0.1169	3600	1.240	0.1771
$1x10^{-4}$	4×10^{-4}	10800	1.213	0.1732			
$1x10^{-4}$	1×10^{-4}	3600	0.262	$0.0324x10^{-4}$	1800	0.245	$0.0350x10^{-4}$
$1x10^{-4}$	1×10^{-4}	3600	0.244	0.0349	1800	0.257	0.0367
$1x10^{-4}$	1×10^{-4}	3600	0.219	0.0313	3600	0.550	0.0786
$1x10^{-4}$	1×10^{-4}	7200	0.461	0.0659	3600	0.518	0.0740
$1x10^{-4}$	1×10^{-4}	7200	0.456	0.0651	7200	0.812	0.1160
$1x10^{-4}$	1×10^{-4}	10800	0.631	0.0901	7200	0.840	0.1200
$1x10^{-4}$	1×10^{-4}	10800	0.627	0.0896	7200	0.794	0.1134
$1x10^{-4}$	1×10^{-4}	10800	0.665	0.0950			
$4x10^{-4}$	1×10^{-4}	3600	0.559	$0.0799x10^{-4}$	1800	0.610	$0.0871x10^{-4}$
$4x10^{-4}$	1×10^{-4}	3600	0.547	0.0781	1800	0.608	0.0869
$4x10^{-4}$	1×10^{-4}	7200	1.088	0.1554	1800	0.611	0.0873
$4x10^{-4}$	1×10^{-4}	7200	1.083	0.1547	3600	1.022	0.1460
$4x10^{-4}$	1×10^{-4}	7200	1.095	0.1564	3600	1.192	0.1702
$4x10^{-4}$	1×10^{-4}	10800	1.629	0.2327	7200	1.880	0.2685
$4x10^{-4}$	1×10^{-4}	10800	1.560	0.2228			

TABLE VIII

Second order rate constants at 115°C

for the formation of nickel complex of pheophytin

	Initial concentration of nickel acetate, mole/litre	Initial concentration of pheophytin, mole/litre	Time, sec	Concentration of nickel complex formed, mole/litre	k litre $mole^{-1}sec^{-1}$
*	1×10^{-4}	4×10^{-4}	1800	0.0909×10^{-4}	0.134
	1×10^{-4}	4×10^{-4}	1800	0.0984	0.113
	1×10^{-4}	4×10^{-4}	1800	0.0866	0.127
	1×10^{-4}	4×10^{-4}	3600	0.1900	0.150
	1×10^{-4}	4×10^{-4}	3600	0.1771	0.138
**	1×10^{-4}	1×10^{-4}	1800	0.0350	0.201
	1×10^{-4}	1×10^{-4}	1800	0.0367	0.212
	1×10^{-4}	1×10^{-4}	3600	0.0786	0.237
	1×10^{-4}	1×10^{-4}	3600	0.0740	0.222
	1×10^{-4}	1×10^{-4}	7200	0.1160	0.182
	1×10^{-4}	1×10^{-4}	7200	0.1200	0.189
	1×10^{-4}	1×10^{-4}	7200	0.1134	0.178
*	4×10^{-4}	1×10^{-4}	1800	0.0871	0.127
	4×10^{-4}	1×10^{-4}	1800	0.0869	0.127
	4×10^{-4}	1×10^{-4}	1800	0.0873	0.127
	4×10^{-4}	1×10^{-4}	3600	0.1460	0.111
	4×10^{-4}	1×10^{-4}	3600	0.1702	0.133
	4×10^{-4}	1×10^{-4}	7200	0.2685	0.113

* Unequal quantities of reactants, values for k were calculated using $k = \frac{1}{t(A-B)} \ln \frac{B(A-x)}{A(B-x)}$

** Equal quantities of reactants, values for k were calculated using $k = \frac{1}{t} \frac{x}{A(A-x)}$

the results of the two systems involving a change in initial nickel acetate concentrations were used in the equation

$$\frac{dx}{dt} = k\ (A-x)^{\alpha}\ (B-x)^{\beta}$$

where $\frac{dx}{dt}$ = rate of formation of the nickel complex over the time period t seconds
k = rate constant
A = initial concentration of nickel acetate
B = initial concentration of pheophytin
x = concentration of complex formed
α = order of reaction with respect to nickel
β = order of reaction with respect to pheophytin

To facilitate solution of the simultaneous equations, the logarithmic expression of each equation was used which gives

$$\log \frac{dx}{dt} = \log k + \alpha \log(A-x) + \beta \log(B-x)$$

In determining the order of reaction in this manner it is obvious that the rate constant, k, is constant and as a result the log k term drops out of both equations on subtraction of one equation from the other. Similarly the concentration values for pheophytin is constant in both equations and will drop out. With these terms taken out the resulting equation will have terms involving $\frac{dx}{dt}$ and the concentration of nickel acetate. The equation for the determina-

tion of the order of reaction with respect to nickel was

$$\log \frac{dx_1}{dt} - \log \frac{dx_2}{dt} = \alpha \log (A_1 - x_1) - \alpha \log (A_2 - x_2)$$

where $\frac{dx_1}{dt}$ = average rate of formation of nickel complex at the higher initial concentration of nickel acetate (4×10^{-4} moles/litre)

$\frac{dx_2}{dt}$ = average rate of formation of nickel complex at the lower initial concentration of nickel acetate (1×10^{-4} moles/litre)

α = order of reaction with respect to nickel

A_1 = initial concentration of nickel acetate at the higher concentration

A_2 = initial concentration of nickel acetate at the lower concentration

x_1 = concentration of complex formed in time t seconds at the higher initial concentration.

x_2 = concentration of complex formed in time t seconds at the lower initial concentration.

A similar equation involving β and B was used to determine the order of reaction with respect to pheophytin.

If we assume that $\frac{\Delta x_1}{\Delta t} = \frac{dx_1}{dt}$ and $\frac{\Delta x_2}{\Delta t} = \frac{dx_2}{dt}$, which is valid for low conversions in the complexing mechanism, values can be calculated for these terms. From the initial concentrations of either nickel acetate or pheophytin and the concentrations of nickel complex formed, the log terms governing those concentrations can be calculated. Solution of the simultaneous equations gives the order of reaction

with respect to the reactant studied.

Values for the order with respect to nickel (α) and pheophytin (β) were determined from the experimental data for each temperature and each time period involved for that temperature. Thirty to sixty calculations were required for the determination of the coefficient for each reactant at each temperature depending on the number of experimental data available. The values calculated for α and β are reported in Table IX. The values found for α showed a minor temperature dependence, while those for β appeared to show only a random variation. Using average values so determined for α and β, the rate expression for the formation of the nickel complex of pheophytin in methanol was found to be

$$\frac{dx}{dt} = k\ (A-x)^{0.7}\ (B-x)^{0.5}$$

for the experimental conditions involved in the present study.

The rate constant, k, was then evaluated from a knowledge of $\frac{dx}{dt}$, A, B and x for all experimental data obtained at each temperature. This involved eighty calculations and the results of the calculations are reported in Table X. In general the values obtained for the rate constant at each temperature showed good agreement, with the standard deviation being about $\pm$ 8.0%. It was clear

TABLE IX

Evaluation of coefficients for nickel (α) and pheophytin (β) concentrations in the rate expression for the formation of the nickel complex of pheophytin

Temp., °C	Coefficient, α	β
74	0.855 ± 0.127	0.476 ± 0.060
90	0.685 ± 0.045	0.456 ± 0.122
103	0.631 ± 0.027	0.465 ± 0.041
115	0.616 ± 0.077	0.662 ± 0.077

TABLE X

Rate constants for the formation of

the nickel complex of pheophytin calculated from

$$\frac{dx}{dt} = k\ (\text{nickel})^{0.7}\ (\text{pheophytin})^{0.5}$$

over the temperature range 74° - 115°C

Concentrations Nickel acetate, mole/litre	Pheophytin, mole/litre	74°C	90°C	103°C	115°C
1×10^{-4}	4×10^{-4}	3.04×10^{-6}	1.83×10^{-5}	5.94×10^{-5}	1.72×10^{-4}
1×10^{-4}	4×10^{-4}	3.19	1.72	6.05	1.47
1×10^{-4}	4×10^{-4}	2.80	1.72	6.20	1.63
1×10^{-4}	4×10^{-4}	3.87	2.10	5.74	1.98
1×10^{-4}	4×10^{-4}	3.83	1.72	5.69	1.82
1×10^{-4}	4×10^{-4}	3.69	1.99	5.86	
1×10^{-4}	4×10^{-4}	3.16	2.04		
1×10^{-4}	1×10^{-4}	2.92×10^{-6}	1.88×10^{-5}	5.90×10^{-5}	1.28×10^{-4}
1×10^{-4}	1×10^{-4}	3.12	1.91	6.40	1.34
1×10^{-4}	1×10^{-4}	3.18	1.89	5.70	1.52
1×10^{-4}	1×10^{-4}	3.21	1.81	6.28	1.39
1×10^{-4}	1×10^{-4}	3.61	1.87	6.18	1.14
1×10^{-4}	1×10^{-4}	3.49	1.62	5.90	1.18
1×10^{-4}	1×10^{-4}		1.69	5.88	1.11
1×10^{-4}	1×10^{-4}		1.69	6.25	
1×10^{-4}	1×10^{-4}		1.65		
4×10^{-4}	1×10^{-4}	3.20×10^{-6}	1.66×10^{-5}	4.91×10^{-5}	1.23×10^{-4}
4×10^{-4}	1×10^{-4}	4.03	1.66	4.89	1.23
4×10^{-4}	1×10^{-4}	3.77	1.66	5.78	1.23
4×10^{-4}	1×10^{-4}	3.32	1.75	5.75	1.08
4×10^{-4}	1×10^{-4}	6.72	1.73	5.82	1.28
4×10^{-4}	1×10^{-4}	4.09		6.14	1.10
4×10^{-4}	1×10^{-4}	5.53		5.83	

that the rate expression so developed fitted the experimental data much more closely than did the second order rate expression.

The Arrhenius equation was evaluated for each set of results, yielding:

1. 1×10^{-4} moles/litre nickel and 4×10^{-4} moles/litre pheopnytin,

$$k = 5.37 \times 10^{10} \quad e^{-25.6 \times 10^{3}/RT}$$

2. 1×10^{-4} moles/litre nickel acetate and 1×10^{-4} moles/litre pheophytin,

$$k = 1.32 \times 10^{9} \quad e^{-23.0 \times 10^{3}/RT}$$

3. 4×10^{-4} moles/litre nickel acetate and 1×10^{-4} moles/litre pheophytin,

$$k = 6.7. \times 10^{9} \quad e^{-24.3 \times 10^{3}/RT}$$

DISCUSSION

Orders of reaction for the formation of the nickel complex of pheophytin from nickel acetate and pheophytin were determined from experimental data. The results indicate orders of 0.7 and 0.5 with respect to nickel acetate and pheophytin respectively yielding the expression

$$\frac{dx}{dt} = k\ (A-x)^{0.7}\ (B-x)^{0.5}$$

The reaction mechanism for the complexing of pheophytin with nickel is obviously not a simple clear-cut second-order mechanism, and it may be worthwhile to consider briefly what mechanisms might be cited to account for the marked deviations from second order behaviour. Two major points should be considered: first, the temperature dependence of the order with respect to nickel acetate, and second, the mechanism which could result in a half order with respect to pheophytin.

The order with respect to nickel acetate trends over the temperature range investigated from about 0.9 at 74°C to about 0.6 at 115°C. The reason for this decrease is not immediately apparent but it is important to note that the rate expression which summarizes the results is only empirical and does not take into consideration the obvious fact that the reaction is fundamentally a replacement of two hydrogen ions by a nickel ion and nickel acetate is not completely dissociated in methanol. All calculations had

been made assuming complete dissociation. Assuming various limited degrees of dissociation, further calculations were made to determine what changes in order with respect to nickel ions, if any, would result from a) incomplete dissociation, and b) changes in degree of dissociation over the temperature range involved.

In considering various limited degrees of dissociation ranging from 70% to 10% it is obvious from the results in Table XI that changes resulting from such variations in the degree of dissociation could not account for the changing order with respect to nickel. Even at 10% dissociation, that is, only 10% of the total nickel present is in the form of ions, the order at 74°C was 0.79 differing only slightly from 0.86 assuming complete dissociation.

No appreciable change in trend of the order was evident as a result of varying the degree of dissociation over the temperature range involved. For example, assume 10% dissociation at 74°C and 70% at 115°C, the trend in order with respect to nickel, 0.79 to 0.60, was not too different from the trend assuming complete dissociation. It is apparent, therefore, that the trend in order with respect to nickel cannot be accounted for by incomplete or variable dissociation of nickel acetate in methanol. This may be accounted for, however, by a reaction mechanism which will be proposed later involving competing and consecutive reactions. The significance of one or more of the

TABLE XI

Order with respect to nickel at various degrees of dissociation of nickel acetate in methanol

Degree of dissociation	74°C	90°C	103°C	115°C
1.00	0.86	0.69	0.63	0.62
0.70	0.85	0.67	0.62	0.61
0.50	0.84	0.66	0.61	0.61
0.30	0.80	0.65	0.60	0.58
0.20	0.79	0.63	0.60	0.57
0.10	0.79	0.62	0.60	0.56

individual steps may be altered with increasing temperature and therefore may account for the trend observed.

The order with respect to pheophytin is about 0.5. While the values obtained for β do not agree exactly, the scatter is probably due to random errors. There is no clearly discernible temperature trend in the order with respect to pheophytin concentration.

It would be expected that the overall reaction system would obey the expression

Nickel acetate + pheophytin $\longrightarrow$ nickel complex + $2H^+$

This reaction indicates an overall second-order rate expression, but the experimental data are not compatible with a second order expression.

A similar incompatability has been observed in the reactions involving the additions of iodine and bromine to hydrogen as summarized by Frost and Pearson (65). The hydrogen-iodine reaction obeyed the second-order rate expression but the hydrogen-bromine reaction did not, because it involved dissociation of bromine molecules into atoms followed by reactions between atoms and molecules. It was obvious that the mechanism could not be predicted from the overall reaction equation alone. Similarly, it is apparent that the mechanism for the formation of the nickel complex of pheophytin probably cannot be predicted from the overall reaction as written.

Hodgson et al (25) observed a dependence on the hydrogen ion concentration in the complexing of pheophytin with nickel in a heterogeneous system; therefore, in proposing a mechanism for the present reaction system, this effect must be considered.

In considering the half order with respect to pheophytin, it is instructive to list some of the more likely important reactions in the overall complexing system. It is not suggested that this is a complete mechanism or the only mechanism, but rather an indication of possible reactions in the overall reaction. It is felt that in order to account for an apparent half order with respect to pheophytin, it is necessary to base the proposed scheme on the formation of an intermediate complex involving one nickel ion and two pheophytin molecules. This immediately suggests a third-order reaction with the order of reaction with respect to pheophytin as second order and not half order as required by the experimental data but a reaction mechanism of consecutive and competing reactions is proposed which may result in a half order with respect to pheophytin.

For the sake of convenience, pheophytin will be represented as PH_2, pheophytin with the hydrogen atoms removed as $P^{=}$ and the nickel complex of pheophytin as NiP.

1. $PH_2 \longrightarrow P^{=} + 2H^{+}$
2. $PH_2 + 2H^{+} \longrightarrow PH_4^{++}$
3. $PH_4^{++} + Ni^{++} \longrightarrow NiPH_2^{++} + 2H^{+}$
4. $2PH_4^{++} + Ni^{++} \longrightarrow Ni(PH_3)_2^{++++} + 2H^{+}$
5. $Ni(PH_3)_2^{++++} + Ni^{++} \longrightarrow 2NiPH_2^{++} + 2H^{+}$
6. $NiPH_2^{++} \longrightarrow NiP + 2H^{+}$
7. $NiPH_2^{++} + 2H^{+} \longrightarrow PH_4^{++} + Ni^{++}$
8. $P^{=} + 2H^{+} \longrightarrow PH_2$

The rate of formation of the nickel complex of pheophytin according to this mechanism is

$$\frac{d(NiP)}{dt} = k\ (NiPH_2^{++})$$

To determine the rate of formation of the nickel complex, the concentration of $NiPH_2^{++}$ must be evaluated in terms of compounds whose concentrations can be determined experimentally, e.g. Ni^{++}, pheophytin and the nickel complex.

The concentration values of certain compounds of the system can be evaluated assuming steady state approximations. In this manner values can be determined for H^{+}, PH_4^{++}, $NiPH_2^{++}$, $Ni(PH_3)_2^{++++}$ and $P^{=}$ in terms of each other. The steady state approximation makes use of the fact that at any time in the course of the reaction the concentration of certain compounds in the reaction remain virtually constant. Using this approximation, the rate of formation of any particular compound is equal to its rate of consumption, or, its overall rate of formation is equal to zero.

The mathematics involved in such calculations is very complex. A complete evaluation of any proposed mechanism requires rate data for each step in the mechanism, proper separation and identification methods for each component and knowledge of the stability of each compound. Such information is not available for the present system and further examination of the mechanism is beyond the scope of the present investigation.

The plot of log k vs $\frac{1}{T}$ in Figure 5 for the determination of the activation energy of the system indicated small, though perhaps significant, departure from linearity. This was thought to be a result of changing order of reaction with respect to nickel ions over the temperature range studied. It was observed earlier that at each temperature, and over a wide range of degrees of dissociation of nickel acetate in methanol, very little variation was found in the order of reaction with respect to nickel ions. Minor changes were noted, however. Calculations were made of rate constants assuming a constant degree of dissociation over the temperature range studied. The resulting rate constants were plotted against $\frac{1}{T}$. No improvement in linearity was observed at constant degree of dissociation and the apparent activation energy was unchanged. Further calculations were made assuming an increase in degree of dissociation with temperature. In the experimental section, the apparent degree of dissociation at room temperature was

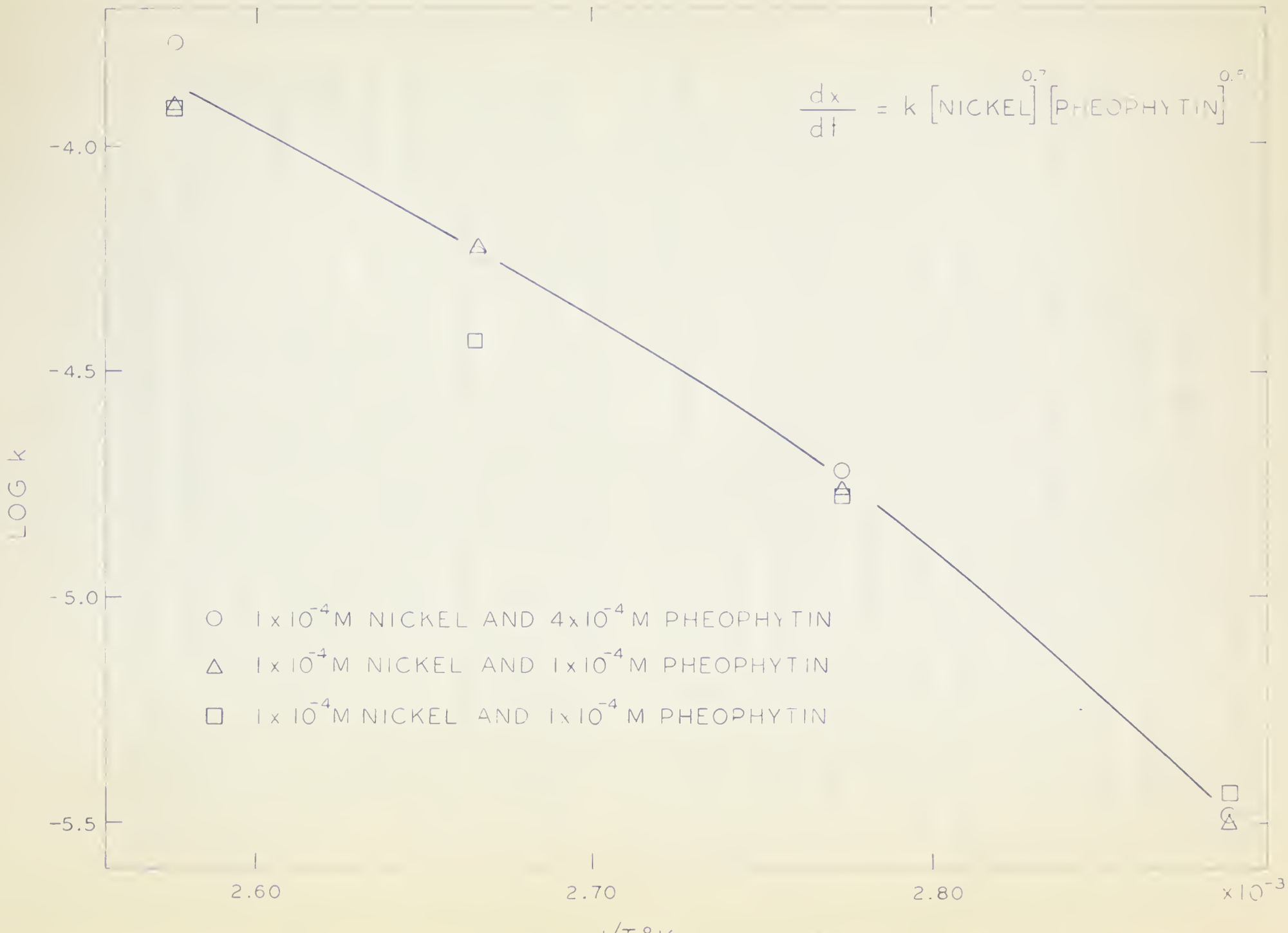


Fig. 5. Activation energy of the rate of formation of the nickel complex of pheophytin.

found to be about 25%. Using this as a guide, extents of dissociation were taken to be 20% at 74 and 90°C and 30% at 103 and 115°C. The plot of log k vs $\frac{1}{T}$ using the new rate constants exhibited an over-correction of the curvature shown in Figure 5 which was based upon complete dissociation of nickel acetate in methanol. A variation of 10% in dissociation indicated an over-correction; therefore it would seem likely that the degree of dissociation did not vary to that extent over the temperature range studied. A further calculation was made with 25% dissociation at 74 and 90°C and 30% at 103 and 115°C. The log k vs $\frac{1}{T}$ plot of these rate constants was virtually a straight line indicating an activation energy of 23.0 kcal/mole and a frequency factor of 3.9×10^9.

The activation energy for the exchange of two hydrogen ions with one nickel ion as derived and modified above should probably be similar to values reported for related exchange reactions. The activation energy for the exchanges of iodide ion with aromatic iodides (66), inorganic iodides with n-butyl iodides (67), dimethyl sulphate with thiocyanate ions (68), ethyl iodide with β-$C_{10}H_7O^-$ (69) and n-propyl iodide with $C_6H_5O^-$ (70) were 25-33, 18.0, 17.9, 21.0 and 22.5 kcal/mole respectively, very similar to the 23.0 kcal/mole obtained in this study.

The frequency factor obtained in the present study,

according to the modified equation, is 3.9×10^9. Bimolecular reactions in solution have a normal or average value of this factor near 10^{11} litres/mole/sec (65). Frequency factors for the exchanges of dimethyl sulphate with thiocyanate ions (68), ethyl iodide with β-$C_{10}H_7O^-$ (69) and n-propyl iodide with $C_6H_5O^-$ (70) were 5.7×10^{10}, 1.0×10^{10} and 3.5×10^{11} respectively. The frequency factor observed in this study is not too different from the above figures and it is reasonable to assume that there is little about the complexing of nickel and pheophytin that sets it apart as an unusual type of system.

GEOCHEMICAL ASPECTS OF THE NICKEL COMPLEX OF PHEOPHYTIN

Extrapolation of the log k vs $\frac{1}{T}$ plot to sedimentary temperatures indicated that the time necessary for 10% conversion of pheophytin to the nickel complex of pheophytin at 10°C, under the conditions studied in the present investigation, would require about three years. This implies that concentration levels of both nickel and pheophytin would be of the same magnitude as those studied here and that the solvent system would be similar to methanol. Under sedimentary conditions there are several features which could alter the overall rate of formation of the nickel complex of pheophytin such as variation in pH, availability of nickel ions, the possible presence of catalysts, the

fact that an aqueous system would be operative and that there is general heterogeneity in the system.

Hodgson et al (25), studying the rate of formation of the nickel complex of pheophytin in recent sediments, examined the complexing reaction in lake muds from North Cooking Lake, Alberta. Since the amount of nickel available for reaction in the muds was very small, a gross excess was added to give measurable amounts of reaction. The mixture was placed in heavy wall glass reaction tubes and the tubes were frozen, evacuated and sealed as in the present study. Heating of the reaction mixture was done in the temperature range 129 - 161°C. Extrapolation of the results to 10°C indicated that the time necessary for significant conversion of pheophytin to the nickel complex was a few decades. From the present study, using the Arrhenius equation and the rate expression derived, and assuming an initial nickel concentration of 1.50×10^{-2} moles/litre and pheophytin concentratiom of 1×10^{-4} moles/litre (to obtain the same working ratios as that above), the time required to convert 10% of the pheophytin to the nickel complex at 10°C is slightly less than one year. If concentrations are used for nickel and pheophytin which have been reported in recent sediments, i.e. about 3×10^{-8} moles/litre for nickel and 4.5×10^{-6} moles/litre for pheophytin, the time required for significant conversion at

the same sedimentary temperature is about 200 years, which is a very minor portion of the 10,000-year period over which the accumulation of recent sediments has taken place in Alberta.

The conclusions of the sediment study (25) and of the present study differ by only about two orders of magnitude in evaluating the time necessary for conversion of pheophytin to the nickel complex. This indicates good general agreement particularly since the extrapolation in the present study is based on an activation energy which probably has an inherent error of $\pm$ 1 kcal/mole. If the activation energy of the reaction studied here were 22 kcal/mole instead of 23 kcal/mole, the time necessary for 10% conversion of pheophytin to its nickel complex would require several decades, very similar to the time reported for the reaction in recent sediments.

The conclusions of the present investigation, indicate that the nickel complex of pheophytin could be formed in recent sediments provided adequate amounts of nickel were available.

SUMMARY

The nickel complex of pheophytin a in diethyl ether is characterized by a bright blue-green color with major absorption maxima at 410 and 635 mu in the visible range. It has a molar extinction coefficient of 7.0×10^4 litres $moles^{-1} cm^{-1}$.

An expression was derived to express the rate of formation of the complex from nickel acetate and pheophytin:

$$\frac{dx}{dt} = k \text{ (Nickel acetate)}^{0.7} \text{ (Pheophytin)}^{0.5}$$

Values for the rate constants in the above equation, for time measured in seconds and concentrations in moles $litres^{-1}$, were 3.7×10^{-6}, 1.8×10^{-5}, 5.9×10^{-5} and 1.4×10^{-4} at 74, 90, 103 and 115°C respectively.

The activation energy of the reaction is given by the equation

$$k = 3.9 \times 10^9 \quad e^{-23.0 \times 10^3/RT}$$

The order of reaction with respect to nickel ions showed a trend over the temperature range studied, decreasing from about 0.9 at 74°C to about 0.6 at 115°C. This observation could not be accounted for by a changing degree of dissociation of nickel acetate in methanol. The order of reaction with respect to pheophytin is apparently half order probably due to the formation of an intermediate

complex involving one nickel ion and two pheophytin molecules in the complex reaction system.

Extrapolation of the log k vs $\frac{1}{T}$ plot to sedimentary temperatures indicated that this reaction is possible in the formation of petroleum porphyrins from chlorophyll in the formation of crude oil, provided that an adequate supply of nickel were available.

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